

State of Hawai'i
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Aquatic Resources
Honolulu, Hawai'i 96813

June 8, 2007

Board of Land
and Natural Resources
Honolulu, Hawai'i

Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National
Monument Research Permit to Dr. Brian Bowen, University of Hawai'i, Hawai'i Institute
of Marine Biology (HIMB), for Access to State Waters to Conduct Reef-Fish Genetics
Research Activities.

The Division of Aquatic Resources (DAR) hereby submits a request for your authorization and approval for issuance of a Papahānaumokuākea Marine National Monument research permit to Dr. Brian Bowen, HIMB, pursuant to § 13-60.5, *Hawaii Administrative Rules*, and § 187A-6, *Hawaii Revised Statutes*, and all other applicable laws and regulations. The research permit, described below, will allow activity to occur in the NWHI State Marine Refuge (0-3 miles) waters surrounding Nihoa Island, Gardner Pinnacles, Laysan Island, Lisianski Island, Pearl and Hermes Atoll, and Kure Atoll. The activities will occur from July 7, 2007 through July 31, 2007, as outlined below and in the attached permit application.

INTENDED ACTIVITIES

The proposed research is a genetic survey of reef fishes, designed to address the issue of population connectivity across the NWHI. The applicant is currently surveying 25-30 species across the entire archipelago, using mitochondrial DNA (mtDNA) sequencing technology. With a few notable exceptions, reef organisms have not been surveyed on this scale.

PROCEDURES

FIELD METHODS

Whenever possible, fish will be sampled non-lethally by removing a rice grain-sized piece of fin (biopsy of less than one square cm) and released in the location from which collected, however most specimens are to be collected with pole spears.

Moray eels and other permitted species will be collected with plastic F-plus eel traps. Moray eels will be collected with this non-lethal methodology, which involves leaving the traps overnight adjacent to reef habitat. Traps will be set at the end of the working day and checked first thing in the morning (about 8:00 – 9:00 am) to minimize stress to

the animals. Statistical rigor requires a minimum sample size of 25 individuals per location

Exceptions to the rule of 25-30 samples per location include the following:

- 1) Moray eels (family Muraenidae) will be limited to 15/location. The eels (and other species where possible) will be trapped live, fin clipped immediately, and released on site.
- 2) The introduced snapper, ta'ape (*Lutjanus kasmira*), will be subjected to both mtDNA and nuclear DNA analyses which require the larger sample size of 50 specimens/location.
- 3) In response to concerns voiced by reviewers in 2006, blenny (family Blenniidae) samples are limited to 15 per expedition.

The applicant's goal is to focus on the species that can be collected rapidly, safely, and without impact to the resident populations. Additionally, the researcher will only be collecting specimens from species that occur in high abundance: i.e., at thousands to millions per atoll. The collections will be spread across an area of approximately five hectares (a maximum of 10 individuals per hectare will be collected so that no one site will be at risk of depletion.

All scuba equipment will be soaked in required disinfection solution between sampling locations, in compliance with decontamination protocols.

LAB METHODS

The primary lab methodology in this study will be sequencing of mtDNA cytochrome genes. In most species, a segment of approximately 800 base pairs of the mtDNA cytochrome *b* or cytochrome oxidase gene will be amplified and sequenced following standard protocols.

REVIEW PROCESS:

The permit application was sent out for review and comment to the following scientific entities: Division of Aquatic Resources staff, Papahānaumokuākea Marine National Monument, NOAA Pacific Islands Regional Office (NOAA-PIRO), and United States Fish and Wildlife Service. The Office of Hawaiian Affairs (OHA), and the Kahoolawe Island Reserve Commission (KIRC) were also consulted.

Comments received from the scientific community and the applicant's written responses are summarized as follows:

Scientific Reviewer's Concerns (reviewer's comments in bullets; responses in italics):

- ⌘ Don't know fish densities at Midway and feel uneasy about allowing take.

Our field experience and collection notes from 2006 demonstrate that fish composition changes slightly between Pearl and Hermes and Midway, but that densities are uniformly high at Pearl and Hermes, Midway, and Kure. As noted in the application, of necessity we target species that are abundant.

- ⌘ More work on live capture and release of fishes. Researchers have made some progress.

Agreed. We diverted research time last year to test nylon mesh traps and hand-nets. The traps worked well in Kaneohe Bay, but were shredded in the more shark-abundant testing ground of Johnston Atoll, as noted in our permit request. The nets, in the hands of experienced fish collector Dr. Jill Zamzow, could capture three to five fish per hour, not sufficient to conduct this research within the logistic parameters of NWHI research cruises. Hence we will test plastic (F plus) traps this year in the NWHI. Tests in Kaneohe Bay demonstrate that these traps may catch morays and other fishes for non-lethal sampling.

- ⌘ Genetic connectivity is different from demographic connectivity.

A decade ago, there was a partial disconnect between genetic and demographic connectivity. If populations were shown to be genetically isolated, they are definitely demographically isolated, so even the earlier genetic studies were valuable. However the converse was not always true. In some cases, populations that were demographically distinct (with low levels of exchange) might not be genetically distinct at detectable levels. In recent years the genetic techniques and computer algorithms have narrowed this gap. With current methodology, it is now possible to assess questions of connectivity on ecological time scales with genetic data. For example, genetic analyses can assign recruits to the population of origin, and these findings are corroborated in tag/recapture studies.

A second perspective comes from the scientific literature. In the most recent issue of the peer-reviewed journal Conservation Genetics (Vol. 8 no. 3), 10 of the 20 major articles use genetic data to infer demographic connectivity. Hence the consensus among peer reviewers is that genetic and demographic connectivity are linked.

A third perspective comes from tagging studies, which are still the best method for resolving demographic processes. It isn't possible to tag and track larvae across the NWHI, so genetic data offers the best scientific alternative to recover this kind of information.

- ⌘ How will directionality of gene flow be determined?

A good point. Two computer programs, MIGRATE and IM use a maximum likelihood algorithm to estimate gene flow between locations in both directions, based on Beerli and Felsenstein (2001, Proceedings of the National Academy of Science 98:4563-4568). This is mentioned in section seven of the permit application but should have been elaborated.

- ⌘ Lots of management hooks here...unclear since demographic connectivity isn't being addressed.

The management focus of the study is generally considered a strong point by reviewers, in keeping with the mandate of the Findings of Presidential Proclamation 8031, particularly Section 3.a.i.A-D concerning the compatibility with management direction of the proclamation.

Demographic and genetic connectivity is addressed two sections above this section.

- ⌘ It is not totally clear which species will be sampled...If this is the case then none of the species should be collected except at Laysan and Lisiansky (sic). This is certainly the case for eels where 15 of each species have already been collected.

The numbers listed in Appendix A (target species) reflect the target sample size (30) for each species minus what has already been collected in previous expeditions. I'm sorry if that wasn't clear. We do not have full sample sizes from everywhere except at Laysan and Lisiansky (sic), and apologize if I gave that impression. In regards to the eels, we were not permitted to collect eels last year, at the recommendation of DAR personnel, and instead experimented with eel traps at Johnston Atoll, outside the monument. Hence we do not have collections of 15 from each eel species/location, and I did not mean to give that impression.

- ⌘ Target species of Craig study should be included in genetic analyses, especially *M. vanicolensis* (yellow goatfish) and *P. meeki* (Hawaiian bigeye).

Agreed. Tissue specimens from all specimens are archived for future genetic analyses, to make maximum use of samples. Currently we do not have funding to add additional species to the genetic analyses, but the specimens will be available when funding for lab expenses is procured.

- ⌘ Collection report should indicate number of mortalities associated with sampling.

Agreed. Prior to 2006, the collections were all mortalities. With the nets and traps, we can distinguish live from lethal collections, and will make that distinction in the next report.

- ⌘ Sampling may not have ecological impacts, but may have behavioral impacts, especially with multi-year sampling.

While sampling fishes, we routinely relocate between dives, to minimize the impact of collections. I should have explained that more thoroughly. We do not intend to conduct

extended multi-year sampling. We can conclude many of the species collections this year, and should have most completed in 2008.

- ⌘ Unclear in Methods whether there will be multiple sampling sites within each location.

Agreed. This is a lapse in my permit request. As mentioned in the section above, we move between collecting sites for every dive, so as to leave the lightest possible footprint.

- ⌘ Non-lethal sampling must be implemented.

We are trying. The permit request outlines our progress and setbacks in this regard, and most reviewers praised our efforts. This year we will test the F-plus traps, and use this non-lethal technology for morays. On the horizon, we are looking at Hawaiian slings with spear heads designed to collect tissue plugs. The tissue plug heads are well developed, based on work with dolphins and other large vertebrates. The key innovation here will be adjusting the spear velocity so that small fish and larger fish can be non-lethally sampled with the same technology. Ultimately we hope that this non-lethal sampling technology will be another valuable conservation tool (and a publication) to come out of this program, initially tested in Kaneohe Bay, and ultimately proven in the NWHI.

- ⌘ All activities cease until monk seals leave the area.

Agreed

Summary:

We are working hard to get the connectivity research done, while having the lightest possible impact on the NWHI. The consensus among reviewers is positive, and I appreciate the scientific acumen, experience, and time that went into these reviews. Hopefully I have responded to the comments in a convincing statement, and will be happy to clear up any remaining ambiguities. The path is clear, and we are all on the same team.

Comments received from the Native Hawaiian community are summarized as follows (reviewer's comments in bullets; responses in italics):

- OHA requests that the research vessel have at least one cultural practitioner on board.

Agreed in principle, but the P.I. does not assign berths on research vessels and can only support this suggestion.

- OHA urges the State to find all applications incomplete (and therefore will not be processed) if they do not include a reference to cultural research, consultation, and resulting protocol.

Agreed. We ask for guidance from OHA on the appropriate consultant for cultural protocols that pertain to the collections of fishes.

RESPONSE:

A meeting was held between HIMB researchers and administrators, and DAR staff, to discuss reviewer's (Scientific, Policy, and Cultural) concerns. The applicant's responses (above) are an outcome of this meeting. The concerns raised by OHA were also discussed at the meeting. It was the consensus of those in attendance, that in order to address the requests from OHA, more information is required from OHA. Specifically, a list of acknowledged cultural practitioners who are available to accompany specific research cruises should be provided by OHA; additionally, a briefing for science researchers should be arranged by OHA or a designate, to provide the information needed by individual applicants in order to address OHA's request for cultural impact analysis.

IMPACT ANALYSIS

Although some disturbances to Monument resources may be necessary to achieve the objective of this project, this research should have minimal impact on Monument resources. The effective management of marine protected areas requires detailed information regarding connectivity among disjunct populations of species. Although this information is needed by the Co-Trustees to effectively manage the resources of the Monument, there is very little information on the relative direction and magnitude of connectivity for any species within the Hawaiian Archipelago. Therefore this study is important in defining the relevant units of management for the Monument and to assess the degree of interconnection among geographically isolated populations of the same species found throughout the Monument jurisdiction. Therefore, the value of this research far outweighs the minimal effects of the research activities.

FINAL STAFF RECOMMENDATIONS:

DAR staff is of the opinion that Applicant has properly demonstrated valid justifications for his application and should be allowed to enter the NWHI State waters and to conduct the activities therein as specified in the application with the following special instructions and conditions, which are in addition to the Papahānaumokuākea Marine National Monument Research Permit General Conditions:

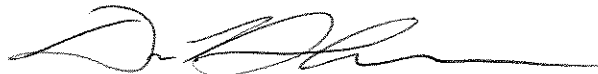
1. Require Applicant to inform and consult with DAR regarding their cruise plan before each trip to the NWHI.
2. Research operations must cease if monk seals are present in the immediate vicinity.
3. No fishing is allowed in State Waters except as authorized under State law for subsistence, traditional, and customary practices by Native Hawaiians.

4. To prevent introduction of disease or the unintended transport of live organisms, the permittee must comply with the disease and transport protocol attached to this permit.
5. Tender and dive vessels operating within the Monument are encouraged to operate at slow speed and with a bow lookout in shallow water coral reef areas in order to minimize prop or bow damage to three dimensional coral reef habitat or endangered monk seals or sea turtles.
6. Tenders and small vessels must be equipped with engines that meet EPA emissions requirements.
7. Refueling of tenders and all small vessels must be done at the NOAA ship Hi'ialakai and outside the confines of the lagoons or near-shore waters.
8. This permit is not to be used for nor does it authorize the sale of collected organisms. Under this permit, the authorized research activity, including work involving a bioassay or bioprospecting, must be for non-commercial purposes, i.e., not involving the use or sale of any organisms, byproducts, or material collected within the Monument for obtaining patents or intellectual property rights for profit.
9. The permittee may not convey, transfer, or distribute, in any fashion (including, but not limited to, selling, trading, giving, or loaning) any coral, live rock, or organism collected under this permit without the express written permission of the Co-Trustees.

RECOMMENDATION:

"That the Board authorize and approve, with stated conditions, a Research Permit to Dr. Brian Bowen."

Respectfully submitted,



DAN POLHEMUS
Administrator

APPROVED FOR SUBMITTAL



ALLAN A. SMITH
Acting Chairperson



Department of Land and Natural Resources
Northwestern Hawaiian Islands Permit Application Review

Permit Type: Management ☐ Recreation ☐ Research ☒
Education ☐ Cultural ☐ Special Ocean Use ☐

Working Title: Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National Monument Research Permit to Dr. Brian Bowen, University of Hawai'i, Hawai'i Institute of Marine Biology (HIMB), for Access to State Waters to Conduct Reef-Fish Genetics Research Activities.

Project Applicant: Dr. Brian Bowen, HIMB

Project Location(s) (Both State Waters and Monument): Nihoa Island, Gardner Pinnacles, Laysan Island, Lisianski Island, Pearl and Hermes Atoll, and Kure Atoll

Project Dates and Duration: July 7, 2007 through September 31, 2007

Project Précis & Background *(Summary of project and why this is proposed):*

The proposed research is a genetic survey of reef fishes, designed to address the issue of population connectivity across the NWHI. The applicant is currently surveying 25-30 species across the entire archipelago, using mitochondrial DNA (mtDNA) sequencing technology. With a few notable exceptions, reef organisms have not been surveyed on this scale.

Are there other relevant permits that have/will be issued with regard to this project? No ☒

What is the relevance to management and/or the improved understanding of NWHI & MHI?

The effective management of marine protected areas requires detailed information regarding connectivity among disjunct populations of species. Although this information is needed by the Co-Trustees to effectively manage the resources of the Monument, there is very little information on the relative direction and magnitude of connectivity for any species within the Hawaiian Archipelago. Therefore this study is important in defining the relevant units of management for the Monument and to assess the degree of interconnection among geographically isolated populations of the same species found throughout the Monument jurisdiction

Could work be conducted outside the NWHI?: Yes ☐ No ☒

Has Applicant been granted a permit from the State in the past? Yes ☒

If so, please summarize past permits:

Permit No. DLNR.NWHI06R004 was issued to the applicant in 2006 for similar research

Have there been any a) violations: Yes ☐ No ☒ **b) late/ incomplete reports:** Yes ☐ No ☒
Any other relevant concerns from previous permits? _____

Recommendations:

DAR Staff: Approve this permit application ☒

Reject this permit application ☐

NH CWG: Approve this permit application ☐

Reject this permit application ☐

Additional Comments:

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Northwestern Hawaiian Islands Marine National Monument
Permit Application

NOTE: This Permit Application (and associated Instructions) are for activities to be conducted in the Northwestern Hawaiian Islands Marine National Monument, including Hawaiian Islands National Wildlife Refuge, the Midway Atoll National Wildlife Refuge, Battle of Midway National Memorial, Northwestern Hawaiian Islands State Marine Refuge, Kure Atoll Hawaii State Seabird Sanctuary, and the Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve. The Co-Trustees are required to determine that issuing the requested permit is compatible with the findings of Presidential Proclamation 8031. Within this Application, please provide all information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historical and cultural resources of the NWHI Marine National Monument (Monument).

Summary Information

Applicant name: Brian Bowen, Ph.D.

Permit categories:

- ☐ Research – Please fill out Sections A-D (as applicable) and Appendix A
- ☒ Conservation and Management - Please fill out Sections A-D (as applicable) and Appendix A
- ☐ Education - Please fill out Sections A-D (as applicable) and Appendix B
- ☐ Native Hawaiian Practices - Please fill out Sections A-D (as applicable) and Appendix C
- ☐ Recreation (Midway ONLY) - Please fill out Sections A-D (as applicable) and Appendix D
- ☐ Special Ocean Use - Please fill out Sections A-D (as applicable) and Appendix E

Briefly describe permit activity:

This project will provide a comprehensive genetic survey of reef-associated fishes to identify stock structure and estimate population connectivity among atolls and banks of the NWHI. This information is needed to define the relevant units of management for the NWHI and to assess the degree of interconnection among geographically isolated populations throughout the Monument jurisdiction. Further, these studies provide the comparison necessary for similar studies in the Main Eight Hawaiian Islands (MHI) to determine whether there is spill-over of individuals (either adults or larvae) from the NWHI into the MHI .

- ☒ This application is for a RENEWAL of an existing permitted project.
- ☐ This application is for a NEW project.

When will the activity take place?

From: June 1, 2007 To: July 15, 2007

NOTE: INCOMPLETE APPLICATIONS WILL NOT BE CONSIDERED

Please Send Permit Applications to:

NWHI Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300

NWHI Monument

Permit Application

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Honolulu, HI 96825

Hoku.johnson@noaa.gov

PHONE: (808) 397-2660 FAX: (808) 397-2662

**NOTE: SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT
REQUIRED. FOR ADDITIONAL SUBMITTAL INSTRUCTIONS, PLEASE SEE PG 7.**

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Bowen, Brian W.

Title: Ph.D., Associate Research Professor, Hawaii Institute of Marine Biology, University of Hawaii. (C.V. available at <http://www2.hawaii.edu/~toonen/bowen.htm>)

2. Mailing address (street/P.O. box, city, state, country, zip):

Hawaii Institute of Marine Biology, 46-007 Lilipuna Road, Kaneohe, HI 96744

Phone: (808) 236-7425

Fax: (808) 236-7433

Email: bbowen@hawaii.edu

For students, major professor's name, telephone and email address:
n/a

3. Affiliation (institution/agency/organization directly related to the proposed project):

University of Hawaii at Manoa, School of Ocean & Earth Science & Technology, Hawaii
Institute of Marine Biology

4. Additional persons to be covered by permit:

Randy Kosaki (Ph.D., MNM), Carl Meyer (Ph.D., HIMB), Robert Toonen (Ph.D., HIMB), Scott Godwin (Ph.D., HIMB), Luiz Rocha (Ph.D., HIMB), Michael Stat (Ph.D., HIMB), Stephen Karl (Ph.D., HIMB), Matthew Craig (Ph.D., HIMB), Jill Zamzow (Ph.D., DAR), Erik Franklin (Researcher, HIMB), Josh Reece (Graduate student, Washington University), Toby Daly-Engel (Graduate student, HIMB), Greg Concepcion (Graduate student, HIMB), Michelle Gaither (Graduate student, HIMB), Yannis Papastamatiou (Graduate student, HIMB)

Additional graduate students may be added if berths become available on the Hi'ialakai – names of these individuals will be forwarded as soon as they are identified.

Section B: Project Information

5a. Project location(s):

<input checked="" type="checkbox"/> Nihoa Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input type="checkbox"/> Necker Island (Mokumanamana)	<input type="checkbox"/> Land-based	<input type="checkbox"/> Ocean-based
<input type="checkbox"/> French Frigate Shoals	<input type="checkbox"/> Land-based	<input type="checkbox"/> Ocean-based
<input checked="" type="checkbox"/> Gardner Pinnacles	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input type="checkbox"/> Maro Reef		
<input checked="" type="checkbox"/> Laysan Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input checked="" type="checkbox"/> Lisianski Island, Neva Shoal	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input checked="" type="checkbox"/> Pearl and Hermes Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input checked="" type="checkbox"/> Midway Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input checked="" type="checkbox"/> Kure Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Ocean-based
<input type="checkbox"/> Other		

NOTE: Please note there is a fee schedule for people visiting Midway Atoll National Wildlife Refuge via vessel and aircraft.

Location Description:

GPS coordinates outlining boundaries for all planned activities are included below:

Location	Longitude	Latitude
Kure Atoll	-178.19706	28.55825
	-178.19624	28.29958
	-178.45988	28.29958
	-178.46071	28.55742
Midway Atoll	-177.19638	28.37420
	-177.19721	28.13377
	-177.52801	28.13460
	-177.52801	28.37420
Pearl and Hermes Atoll	-176.08851	28.04643
	-175.63289	28.04540
	-175.63289	27.70729
	-176.08954	27.70626
Lisianski Island	-173.67293	26.25151
	-173.67293	25.83943
	-174.23095	25.83943
	-174.23095	26.25151
Laysan Island	-171.47900	25.96027
	-171.47725	25.65597
	-171.97918	25.65772
	-171.97918	25.96202
Gardner Pinnacles	-167.74832	25.26071
	-167.75087	24.34878
	-168.36222	24.35133
	-168.36477	25.26071

	-161.66032	23.23817
	-161.66287	22.94013
Nihoa Island	-162.05005	22.94268
	-162.05260	23.23562

5b. Check all applicable regulated activities proposed to be conducted in the Monument:

- ☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving monument resource
- ☐ Drilling into, dredging, or otherwise altering the submerged lands other than by anchoring a vessel; or constructing, placing, or abandoning any structure, material, or other matter on the submerged lands
- ☒ Anchoring a vessel
- ☐ Deserting a vessel aground, at anchor, or adrift
- ☐ Discharging or depositing any material or matter into the monument
- ☒ Touching coral, living or dead
- ☒ Possessing fishing gear except when stowed and not available for immediate use during passage without interruption through the monument
- ☐ Attracting any living monument resource
- ☐ Sustenance fishing (Federal waters only, outside of Special Preservation Areas, Ecological Reserves and Special Management Areas)
- ☐ Subsistence fishing (State waters only)
- ☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

6. Purpose/Need/Scope *State purpose of proposed activities:*

The proposed research is a genetic survey of reef fishes, designed to address the issue of population connectivity across the NWHI. We will survey 25-30 species across the entire archipelago, using mtDNA sequencing technology. With a few notable exceptions (e.g. Rivera et al. 2004) reef organisms have not been surveyed on this scale.

Management need: An ongoing issue for management of the NWHI is whether this is a series of relatively fragile (isolated) ecosystems, or whether it is a single robust ecosystem. There is also a concern about whether the NWHI serves as a source of larvae to replenish depleted fisheries in the main Hawaiian Islands. The assays of population connectivity outlined here will address these issues in a format that has statistical power and scientific credibility.

The purpose of the proposed research is to define the level of isolation among reef communities in the NW Hawaiian archipelago. How fragile are the geographically isolated reef habitats of the NWHI? If these habitats are highly connected by larval dispersal, then any one of them can recover quickly from human or natural perturbation. If they are isolated, they have to recover without significant input from other islands and atolls.

Objectives: The objective of this permit request is a genetic (mtDNA) survey fish species across the NWHI to assess the level of connectivity among isolated reef habitats. We can accomplish this with samples of up to 30 fish specimens/species/location. Each location is defined as an atoll or reef, and collections will be made at a low density of approximately 10 individuals per hectare. The target species are chosen to be abundant and widespread in the archipelago, easy to identify, and easy to collect. Every effort is made to minimize the impact of these collections on the natural communities.

To preserve biodiversity, it is important to know how it arises (Bowen and Roman 2005). While the main objective is to assess genetic connectivity among shallow reef habitats, a “value added” component is that we can assess the age and origin of Hawaiian fauna as well as the age and origins of populations on each island. A genealogical approach to relationships among mtDNA haplotypes, including statistical parsimony (Templeton et al. 1992, Clement et al. 2000) will indicate whether the closest relatives to the Hawaiian fauna lie predominantly to the West (Ogasawara Arch, Wake Island, or Marshall Islands) or to the South (*Johnston Atoll*, Line Islands; Gosline 1955; Maragos and Jokiel 1986; Maragos et al. 2004). In these cases, populations of the widespread Indo-Pacific species will be compared to the Hawaiian endemic. The geographic source of the Hawaiian form (especially Hawaiian endemics) will be resolved with parsimony networks and phylogenetic tools (see Methods), and the age of colonization events will be estimated with the mtDNA molecular clock.

Reef fauna typically have a pelagic phase (eggs and larvae), which lasts 20-60 days, followed by settlement onto a reef where they remain through juvenile and adults stages. Long distance dispersal is accomplished almost exclusively during the pelagic larval phase. However, the geographic limits of such dispersal are uncertain (Bowen et al. 2006a; 2006b). Recent research shows that effective dispersal of marine larvae can fall short of their potential (Swearer et al. 2002). These findings set the stage for a methodical range-wide survey of reef faunas in the Hawaiian archipelago.

Management benefits: These data will provide information on connectivity required for management, and can also detect cryptic endemic species and document the patterns and history of species entering the Hawaiian Archipelago. Furthermore, by documenting the pattern and magnitude of connectivity in a diverse set of fish taxa, we can determine if there are general patterns that can guide management decisions for understudied species. The genetic surveys of connectivity among reef habitats will substantially augment the scientific foundation for conservation measures. Specifically, this research will establish whether reef ecosystems of the NW Hawaiian Islands are isolated management units (as preliminary data indicate) or components of an interactive metapopulation. In the former case, each reef ecosystem will have to recover from environmental insults (whether human or natural) without significant input from other reef ecosystems. A corresponding conservation mandate would be that each ecosystem is an independent management unit.

Justification

The proposed research is a multispecies approach to assess population connectivity among tropical reef habitats of the Hawaiian archipelago. The immediate motivation for this work is to bolster the scientific foundations for comprehensive protection and conservation of the native marine resources in the Northwest Hawaiian Islands. These activities are consistent with management goals of the U.S. Fish and Wildlife Service National Wildlife Reserves.

Connectivity studies are mandated for the Marine National Monument in order to assist decision making and conservation programs. This information will also be critical to assessing the patterns and magnitude of connection between NWHI and the main Hawaiian Islands.

The utility of this information for resolving dispersal pathways is readily apparent. Population connectivity across the NWHI is an essential foundation for prudent management and the establishment of no-take zones throughout the entire Hawaiian Archipelago and beyond. These data are also essential to the on-going debate on whether the NWHI serve as a source or sink for fisheries species in the Main Hawaiian Islands.

7. As explained further in the instructions, please provide any information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historical and cultural resources of the Monument:

Our proposed activities are compatible with the conservation and management goals of the MNM, NWR, and Hawaii state reserve, as outlined below.

A) The proposed research is designed for minimal impact, focused on common species which occur in large numbers. For example, these species are consumed by ulua and other large predators at the rate of 30,000 tons per year at French Frigate Shoals (Sudecum et al. 1991; Friedlander and DeMartini 2002). Hence the take of 15-30 specimens (50 for the introduced ta'ape) per location will have an undetectable impact.

Our ongoing goal is develop (and in some cases pioneer) technology for nonlethal sampling of fishes. Particularly in response to reviewer concerns about the low density of moray eels, we have spent the last year testing traps to catch eels and other fishes for nonlethal sampling. Our first set of nylon twine/steel frame traps were destroyed by sharks within minutes after we set them (Fig. 1). However, our second set employed the F-



Figure 1. Shark concluding our first attempt at nonlethal sampling.

plus plastic trap with much greater success. These traps have been tested rigorously around HIMB, where we have managed to collect over two dozen eels without a single injury to eel or researcher. We propose the eel traps as the sole method for procuring moray eel specimens, and will attempt to trap additional species with this nonlethal methodology.

We also tested nets for nonlethal sampling during the 2006 NWHI collecting trip, with less success. Relying on the considerable expertise of Dr. Jill Zamzow, we found that net trapping was much less efficient than pole spearing, although we will continue to use this methodology where possible for some of the small fish such as blennies (Family Blenniidae).

In sum, we make every effort to maximize management benefits, and minimize negative impacts to the system, including decontamination between locations as outlined in the **Procedures** below. We believe that we have implemented every reasonable safeguard for the resources and ecological integrity of the Monument in our research, and we do not expect any detectable impact from our research sampling.

B) We are fully compliant with conditions described in the Findings of Presidential Proclamation 8031, particularly Section 3.a.i.A-D concerning the compatibility with management direction of the proclamation. These concerns also include ecological integrity and minimal impact.

C) There is no alternative methodology to assessing connectivity between reef habitats in the NWHI. Clearly we have to sample habitats within the NWHI to resolve connectivity in this region.

D) The end value of the research clearly outweighs the imperceptible impacts from our sampling. As described in Section 7A above, **our collections of a few kilograms of fish per location stand against the tens of thousands of tons harvested naturally by apex predators** (Sudekum et al. 1991; Freidlander and DeMartini 2002). In contrast, reef connectivity data will have a direct impact on the assessment of hazards for atolls and islands of the NWHI. Indeed, **the connectivity issue is identified as an essential foundation for reef management** in the pages of the prestigious journal Science (Dawson et al. 2006).

E) The cruise length is shorter than needed to complete field efforts, and so the proposed collections, which were begun in 2005, will extend until 2008. Specimens will be collected across multiple cruises and multiple years.

F) Bowen will be the lead the project, with 20 years experience in this field, including two prior expeditions to the NWHI. He is known to the MNM staff, DAR staff, and USFWS staff, and is clearly qualified to perform this research.

G) There are sufficient financial resources in NWHIMNM-HIMB partnership to conduct and complete the research outlined herein. The partnership is supplemented by two grants from the National Science Foundation to Bowen and Rob Toonen.

H) The genetic methods outlined herein have been employed by Bowen and colleagues in over 70 peer-reviewed publications, and are widely recognized as appropriate for the proposed activity. The fact that both Toonen and Bowen have been awarded highly-competitive NSF grants to expand these activities speaks to the quality of the research. The use of genetic sampling is widely regarded as the cheapest and most robust way in which to answer questions of connectivity on these scales.

I) The NOAA vessel Hi'ialakai is properly outfitted with the OLE approved transceiver and complies with requirements of Presidential Proclamation 8031.

J – There are no other factors that would make the issuance of the permit inappropriate

8. Procedures:

FIELD METHODS

The fish species listed in Appendix A inhabit shallow reefs and are accessible via snorkeling and scuba dives to depths of less than 100 feet. Whenever possible, we sample non-lethally and remove a rice-grain sized piece of fin (biopsy of less than one square cm) and release the animal in the location from which it was collected. We have made significant progress in nonlethal sampling (see Section 7-A above), however most specimens are collected with polespears.

Moray eels and other permitted species will be collected with plastic F-plus eel traps, using methodology developed by Josh Reece, Brian Bowen, and colleagues over the last year. Moray eels will only be collected with this nonlethal methodology, which involves leaving the traps overnight adjacent to reef habitat. Traps will be set at the end of the working day (about 4:00 am) and checked first thing in the morning (about 8:00 – 9:00) am to minimize distress to the animals.

On the proposed expedition, specimens will be collected at Nihoa, Gardner Pinnacles, Lisianski and/or Laysan (weather permitting), Pearle and Hermes, Midway, and Kure. These will be compared to specimens collected previously in the main Hawaiian Islands, Nihoa, French Frigate Shoals, Pearl and Hermes, Midway, Kure, and Johnston Atolls.

Statistical rigor requires a minimum sample size of 25 individuals per location. In studies examining the statistical power for inferring connectivity based on molecular tools, Ruzzante (1998) showed that sample sizes of less than 30-50 had significant bias and could be misleading. Therefore, in the interest of maintaining statistical rigor while minimizing the number of samples collected, our target sample size is 30/location for most fish species.

Exceptions to the rule of 30 per location include the following:

- 1) Moray eels (family Muridae) will be limited to 15/location, and we are likely to fall short of that for most eel species due to lower density. Notably, the eels (and other species where possible) will be trapped live, fin clipped immediately, and released on site.
- 2) The introduced ta'ape (*Lutjanus kasimira*) will be subjected to both mtDNA and nuclear DNA analyses which require the larger sample size of 50 specimens/location.
- 3) In response to concerns voiced by reviewers in 2006, blenny (family Blenniidae) samples are limited to 15 per expedition.

Given that we have two to five days of collecting per location, our goal will be to focus on the species that can be collected rapidly, safely, and without impact on the resident populations. Likely collection sites at each location have been identified by Randy Kosaki, and were also scouted by our researchers on the June 2006 cruise. However, we require some flexibility because our priorities for collecting at specific locations will be dictated in part by unpredictable field conditions and weather. As noted elsewhere, we are only collecting specimens from species that occur in high abundance: i.e., at thousands to millions per atoll. Also the collections will be spread across an area of approximately five hectares (we will collect a maximum of 10 individuals per hectare, much less for moray eels), so that no one site will be at risk of depletion.

All scuba equipment is soaked in a bleach solution between sampling locations, in compliance with decontamination protocols.

LAB METHODS

The primary lab methodology in this study will be sequencing of mtDNA cytochrome genes. In most species, a segment of approximately 800 base pairs of the mtDNA cytochrome *b* or cytochrome oxidase gene will be amplified and sequenced following protocols used daily in our laboratory. DNA sequences will be generated with an ABI 3100 automated DNA sequencer in our lab. Genomic DNA aliquots will be maintained in long-term storage at HIMB so that the genetic material collected will be available for future studies.

Advances in population genetics, especially coalescence theory, will greatly enhance our analysis, elucidating the history of reef organisms, including the effective population size, founder events, and patterns of population collapse and recovery (Harpending et al. 1998, Beerli and Felsenstein 2001, Emerson et al. 2001).

DNA sequence variation will be summarized with standard diversity indices and with an analysis of molecular variance (AMOVA) using ARLEQUIN vers. 2 (Schneider et al. 2000). Phylogenetic methods will include neighbor joining and maximum likelihood algorithms in PAUP version 4.0 (Swofford 2002). Population separations will be defined with using F_{st} values and the maximum likelihood approach of MIGRATE vers. 1.7.3 (Beerli and Felsenstein 2001).

PERSONNEL

All permit personnel listed in Section A-4 are employees of HIMB, with the exception of Josh Reece (employed at Washington University), Jill Zamzow (DAR), and Randall Kosaki (MNM). We do not require the assistance of DAR, FWS, or MNM personnel to complete the proposed activity. However we note that Jill Zamzow (DAR), Jim Maragos (FWS) and Randy Kosaki (MNM) have been very supportive and have directly assisted field efforts.

Section C: Logistics

9. Other permits (list and attach documentation of all other related Federal or State permits):

- 2005 NWHI Coral Reef Ecosystem Reserve Permit
USFWS Pacific Remote Island National Wildlife Refuge Special Use Permit
- 2006 NWHI Coral Reef Ecosystem Reserve Permit
NWHI Marine National Monument Permit
USFWS Pacific Remote Island National Wildlife Refuge Special Use Permit
State of Hawaii Marine Refuge Special Use Permit

9a. For each of the permits listed, please identify any permit violations or any permit that was suspended, amended, modified or revoked for cause. Please explain the circumstances surrounding the violation or permit suspension, amendment, modification or revocation.

None

10. Funding sources (Please attach copies of your budget, specific to proposed activities under this permit and include funding sources. Please see instructions for more information):

Detailed budget information is available upon request from the Monument Permit Coordinators, and sufficient funding exists to complete the research outlined herein. This research is currently, or has been previously, funded by a combination of the following agency sources:

- 1) NWHIMNM-HIMB partnership
- 2) National Science Foundation
- 3) Hawaii Coral Reef Initiative
- 4) Hawaii Sea Grant

11. Time frame:

Activity start: 2005

Activity completion: 2010

Dates actively inside the Monument:

From: ~8 June, 2007

To: ~28 June, 2007

Please describe any limiting factors in declaring specific dates of the proposed activity at the time of application:

Expedition dates are somewhat fluid so we have asked that the permit be issued for June 1 – July 15, 2007. We are awaiting final word from the NOAA vessel Hi'ialakai regarding the exact dates of departure and return.

Personnel schedule in the Monument:

All personnel will be at the same location as dictated by the travel schedule of the NOAA vessel Hi'ialakai. The tentative 2007 June cruise schedule is:

08 June 2007 Depart Honolulu 0800, transit to Nihoa (245 NM)
09 June 2007 Arrive Nihoa, full day dive ops
10 June 2007 Full day dive ops at Nihoa, Depart 1830 for Gardner Pinnacles (355 NM)
11 June 2007 Transit
12 June 2007 Arrive Gardner 0600, full day diving, depart 1830 for Lisianski (335 NM)
13 June 2007 Transit
14 June 2007 Arrive Lisianski 0600, full day dive ops
15 June 2007 Full day dive ops at Lisianski, Depart 1830 for Pearl & Hermes (256 NM)
16 June 2007 Arrive Pearl & Hermes 0730, full day dive ops
17 June 2007 Full day dive ops at Pearl & Hermes, Depart 1830 for Kure (135 NM)
18 June 2007 Arrive Kure 0730, full day dive ops
19 June 2007 Full day dive ops at Kure, Depart 1930 for Midway (60 NM)
20 June 2007 Arrive Midway early AM, 2/3 day dive ops
21 June 2007 2/3 day dive ops at Midway, Depart 1830 for Laysan (335 NM)
22 June 2007 Transit
23 June 2007 Arrive Laysan 0600, full day dive ops
24 June 2007 Full day dive ops at Laysan, Depart 1830 for Honolulu (810 NM)
25 June 2007 Transit
26 June 2007 Transit
27 June 2007 Transit
28 June 2007 Arrive Honolulu early AM

12. Please indicate (with attached documentation) what insurance policies, bonding coverage, and/or financial resources are in place to pay for or reimburse the Monument trustees for the necessary search and rescue, evacuation, and/or removal of any or all persons covered by the permit from the Monument:

All scientific personnel aboard the NOAA vessel Hi'ialakai will be covered by insurance from their respective institutions, as indicated in Section A-4. The majority are covered by University of Hawaii.

13. Please check the appropriate box to indicate how personnel will enter the Monument:

- ☒ Vessel
☐ Aircraft

Provide Vessel and Aircraft information:
NOAA Vessel Hi'ialakai

14. What certifications/inspections do you have scheduled for your vessel? Please fill in scheduled date (attach documentation):

- ☐ Rodent free, Date:
☐ Tender vessel, Date:
☐ Ballast water, Date:
☐ Gear/equipment, Date:
☐ Hull inspection, Date:

15. Vessel information (NOTE: if you are traveling aboard a National Oceanic and Atmospheric Administration vessel, skip this question):

Vessel name:
Vessel owner:
Captain's name:
IMO#:
Vessel ID#:
Flag:
Vessel type:
Call sign:
Embarkation port:
Last port vessel will have been at prior to this embarkation:
Length:
Gross tonnage:
Total ballast water capacity volume (m3):
Total number of ballast water tanks on ship:
Total fuel capacity:
Total number of fuel tanks on ship:
Marine Sanitation Device:
Type :

How will you comply with the 'No Discharge' regulations stipulated in Presidential Proclamation 8031? Describe in detail. If applicable, please attach schematics of the vessel's discharge and treatment systems:

Other fuel/hazardous materials to be carried on board and amounts:

Please provide proof of a National Oceanic and Atmospheric Administration (NOAA) Office of Law Enforcement-approved Vessel Monitoring System (VMS). Please provide the name and contact information of the contractor responsible for installing the VMS system. Please also describe unit name and type:

VMS Email:

Inmarsat ID#:

16. Tender information:

On what workboats (tenders) will personnel, gear and materials be transported within the Monument? Please list the number of tenders/skiffs aboard and specific types of motors:

The Hi'ialakai tender vessels include:

HI-1 (8m) in-board diesel jet

HI-2 (10m) in-board diesel jet

Inflatable craft and Boston whaler with 60 hp outboard motor

Section D: Additional Information for Land Based Operations

17. Proposed movement of personnel, gear, materials, and, if applicable, samples:

18. Room and board requirements on island:

19. Work space needs:

With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury of that the information I have provided on this application form is true and correct.

Signature

Date

PLEASE SEND ONE SIGNED APPLICATION VIA MAIL TO THE MONUMENT OFFICE BELOW:

NWHI Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
FAX: (808) 397-2662

DID YOU INCLUDE THESE?

- ☒ Applicant CV/Resume/Biography
- ☒ Electronic and Hard Copy of Application with Signature
- ☐ Map(s) or GPS point(s) of Project Location(s), if applicable
- ☐ Funding Proposal(s)
- ☐ Funding and Award Documentation, if already received
- ☐ Documentation of Insurance, if already received
- ☐ Documentation of Inspections
- ☐ Documentation of all required Federal and State Permits or applications for permits
- ☐ Statement of information you wish to be kept confidential

Appendix A: Research OR Conservation and Management Application

NOTE: If land or marine archeological activities are involved, please contact the Monument Permit Coordinator at the address on the general application form before proceeding, as a customized application will be needed. For more information, please contact the Monument office on the first page of this application.

1a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name: See Appendix 1

Scientific name:

& size of specimens:

Collection location:

☒ Whole Organism ☒ Partial Organism

Sample sizes reflect the collections already made at Nihoa, Gardner, Pearl and Hermes, Midway, and Kure. No collections were previously made at Laysan and Lisianski.

Common name	Scientific name	No., Size, Locations
Family Acanthuridae		
Yellow tang	<i>Zebrasoma flavescens</i>	8 all sizes Nihoa 30 all sizes Gardner Pinnacles 30 all sizes Laysan 30 all sizes Lisianski 0 all sizes Pearl and Hermes 0 all sizes Midway 30 all sizes Kure
Brown surgeonfish	<i>Acanthurus nigrofuscus</i>	6 all sizes Nihoa 1 all sizes Gardner Pinnacles 30 all sizes Laysan 30 all sizes Lisianski 20 all sizes Pearl and Hermes 29 all sizes Midway 23 all sizes Kure
Blue-lined surgeonfish	<i>Acanthurus nigroris</i>	17 all sizes Nihoa 6 all sizes Gardner Pinnacles 30 all sizes Laysan 30 all sizes Lisianski 0 all sizes Pearl and Hermes 3 all sizes Midway 30 all sizes Kure

Orangecheek surgeonfish *Acanthurus olivaceus* 4 all sizes Nihoa
 12 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 0 all sizes Pearl and Hermes
 19 all sizes Midway
 27 all sizes Kure

Goldring bristletooth *Ctenochaetus strigosus* 6 all sizes Nihoa
 0 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 0 all sizes Pearl and Hermes
 0 all sizes Midway
 0 all sizes Kure

Family Chaetodontidae

Oval butterflyfish *Chaetodon lunulatus* 30 all sizes Nihoa
 30 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 0 all sizes Pearl and Hermes
 0 all sizes Midway
 17 all sizes Kure

Milletseed butterflyfish *Chaetodon miliaris* 30 all sizes Nihoa
 5 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 5 all sizes Pearl and Hermes
 12 all sizes Midway
 8 all sizes Kure

Blueline butterflyfish *Chaetodon fremblii* 23 all sizes Nihoa
 17 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 30 all sizes Pearl and Hermes
 30 all sizes Midway
 30 all sizes Kure

Pebbled butterflyfish *Chaetodon multicinctus* 16 all sizes Nihoa
 21 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 10 all sizes Pearl and Hermes
 0 all sizes Midway
 0 all sizes Kure

Family Labridae

Ornate wrasse *Halichoeres ornatissimus* 24 all sizes Nihoa
 28 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski

5 all sizes Pearl and Hermes
 20 all sizes Midway
 27 all sizes Kure

Yellowtail coris *Coris gaimard*

30 all sizes Nihoa
 30 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 29 all sizes Pearl and Hermes
 17 all sizes Midway
 30 all sizes Kure

Slow wrasse *Coris venusta*

29 all sizes Nihoa
 29 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 5 all sizes Pearl and Hermes
 12 all sizes Midway
 24 all sizes Kure

Blacktail wrasse *Thalassoma ballieui*

29 all sizes Nihoa
 29 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 0 all sizes Pearl and Hermes
 23 all sizes Midway
 0 all sizes Kure

Family Blenniidae

Scarface blenny *Cirripectes vanderbilti*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Fangblenny *Plagiotremus goslinei*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Fangblenny *Plagiotremus ewaensis*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Family Lutjanidae

Blueline snapper (ta'ape) *Lutjanus kasmira*

41 all sizes Nihoa
 50 all sizes Gardner Pinnacles

50 all sizes Laysan
 50 all sizes Lisianski
 50 all sizes Pearl and Hermes
 24 all sizes Midway
 30 all sizes Kure

Family Mullidae

Manybar goatfish *Parupeneus multifasciatus*

30 all sizes Nihoa
 30 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 10 all sizes Pearl and Hermes
 23 all sizes Midway
 19 all sizes Kure

Family Serranidae

Peacock hind (roi) *Cephalopholis argus*

30 all sizes Nihoa
 30 all sizes Gardner Pinnacles
 30 all sizes Laysan
 30 all sizes Lisianski
 30 all sizes Pearl and Hermes
 30 all sizes Midway
 30 all sizes Kure

Family Muraenidae (NOTE: Nonlethal sampling with eel traps)

Snowflake moray *Echidna nebulosa*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Zebra moray *Gymnomuraena zebra*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Yellowfin moray *Gymnothorax flavimarginatus*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Steindachner's moray *Gymnothorax steindachneri*

15 all sizes Nihoa
 15 all sizes Gardner Pinnacles
 15 all sizes Laysan
 15 all sizes Lisianski
 15 all sizes Pearl and Hermes
 15 all sizes Midway
 15 all sizes Kure

Whitemargin moray *Gymnothorax albimarginatus*

15 all sizes Nihoa

	15 all sizes Gardner Pinnacles
	15 all sizes Laysan
	15 all sizes Lisianski
	15 all sizes Pearl and Hermes
	15 all sizes Midway
	15 all sizes Kure
Whitemouth moray <i>Gymnothorax meleagris</i>	15 all sizes Nihoa
	15 all sizes Gardner Pinnacles
	15 all sizes Laysan
	15 all sizes Lisianski
	15 all sizes Pearl and Hermes
	15 all sizes Midway
	15 all sizes Kure
Undulated moray <i>Gymnothorax undulates</i>	15 all sizes Nihoa
	15 all sizes Gardner Pinnacles
	15 all sizes Laysan
	15 all sizes Lisianski
	15 all sizes Pearl and Hermes
	15 all sizes Midway
	15 all sizes Kure
Stout moray <i>Gymnothorax eurostus</i>	15 all sizes Nihoa
	15 all sizes Gardner Pinnacles
	15 all sizes Laysan
	15 all sizes Lisianski
	15 all sizes Pearl and Hermes
	15 all sizes Midway
	15 all sizes Kure

Opportunistic Sampling of Apex Predators

We request authority to collect and archive tissue specimens of less than one gram weight from the apex predators captured during tagging studies by Carl Meyer and colleagues. In the course of placing acoustic and satellite tags, a small piece of tissue is dislodged from the fish, and we seek to archive these under the mandate to make maximum use of sampling opportunities. **Under no circumstances will these species be subject to directed fishing effort.** Possible capture species include, but are not limited to:

Tiger shark	<i>Galeocerdo cuvier</i>
Galapagos shark	<i>Carcharhinus galapagensis</i>
Grey reef shark	<i>Carcharhinus amblyrhynchos</i>
Blacktip shark	<i>Carcharhinus melanopterus</i>
Whitetip reef shark	<i>Triaenodon obesus</i>
Jack (Ulua)	<i>Caranx spp.</i>
Green jobfish	<i>Aprion virescens</i>
Wahoo (Ono)	<i>Acanthocybium solandri</i>
Barracuda	<i>Sphyraena barracuda</i>
Tuna	<i>Thunnus spp.</i>

1b. What will be done with the specimens after the project has ended?

Preserved tissue samples suitable for DNA work will be archived at HIMB for future permitted uses. PI Bowen will be responsible for the database which will track each sample and will be the lead contact for persons wishing to access the tissue sample collection. No samples will be provided to researchers outside HIMB without prior consent of Permit Coordinators.

1c. Will the organisms be kept alive after collection? ☐ Yes ☒ No

- Specific site/location:
- Is it an open or closed system? ☐ Open ☐ Closed
- Is there an outfall? ☐ Yes ☐ No
- Will these organisms be housed with other organisms? If so, what are the other organisms?
- Will organisms be released?

2. If applicable, how will the collected samples or specimens be transported out of the Monument?

NOAA vessel Hi'ialakai

3. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:

It is incumbent on us to make **maximum use of specimens**, especially when they are derived from lethal collections. Towards this end, we have coordinated fish species lists with a parallel project by Erik Franklin and Matt Craig. They can use the specimens collected initially for genetic analysis to resolve age, growth, diet, and other aspects of natural history. Further, the nonlethal sampling of apex predators (listed in Appendix 1) will make maximum use of the captures conducted by Carl Meyer and colleagues. We previously provided specimens for the Bishop Museum, for ciguatera research at University of Hawaii at Hilo, and for life history studies. We will continue this effort to make the most of precious specimens collected in the NWHI.

An electronic database of all samples will be available upon completion of the studies outlined here. This database will be searchable against future permit requests and can reduce the need for return trips to collect tissue samples in the NWHI, and prevent duplicative sampling efforts.

4a. Gear and materials:

Materials include snorkel and scuba gear (mask, fins, snorkel, wetsuit, tank, BCD), collection bag, polespear, hand nets, eel traps, and a high resolution digital camera in an underwater housing to photo-document the collections.

4b. Please list all Hazardous Materials you propose to take to and use within the Monument:

Tissue preservative solutions for DNA analyses include: 95% ethanol (EtOH), MSDS attached, and saturated salt buffer with dimethylsulfoxide (DMSO), MSDS attached. Both EtOH and DMSO are commonly sold for human consumption, and should not pose a significant health or environmental risk.

5. Fixed installations and instrumentation:

None

6. Provide a time line for sample analysis, data analysis, write-up and publication of information:

We expect sampling to be mostly complete in 2008, then analysis of samples is usually completed within roughly a year. Data analysis and write-up usually take no more than an additional year, although the turn-around time for some journals can exceed 300 days, so time to publication can still be considerable post-submission of the study.

Results from these studies are made available to Monument, FWS, and stat managers as quickly as possible. Monthly brown-bag luncheons at HIMB allow researchers to highlight important or interesting new results and discuss them with the management personnel. In addition, we hold biannual symposia during which researchers present the most current findings from their ongoing research in the Monument. These efforts ensure that research results are provided to the Monument co-trustees almost as quickly as they become available.

7. List all publications directly related to the proposed project:

Craig, M.T., J.A. Eble, D.R. Robertson, B.W. Bowen. 2007. High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Marine Ecology Progress Series In press*

Schultz, J.K., R.L. Pyle, E. DeMartini, and B.W. Bowen. 2007. Genetic homogeneity among color morphs of the flame angelfish, *Centropyge loriculus*. *Marine Biology In press*

DID YOU INCLUDE THESE?

☒ Material Safety Data Sheets for Hazardous Materials

Appendix B: Education Application

1. Are you collaborating with others in any way to reduce duplicative activities in the Monument or elsewhere?

2. Gear and materials:

3. Fixed installations and instrumentation:

4. Is your proposed activity based on a State Department of Education Standards Based Curriculum? If so, please describe:

5. What materials, products or deliverables will be developed as a result of your proposed activity? Provide a time line for write-up and publication of information or production of educational materials:

6. List all publications/references directly related to the proposed project:

7a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name:

Scientific name:

& size of specimens:

Collection location:

☐ Whole Organism ☐ Partial Organism

7b. What will be done with the specimens after the project has ended?

7c. Will the organisms be kept alive after collection? ☐ Yes ☐ No

• Specific site/location:

• Is it an open or closed system? ☐ Open ☐ Closed

• Is there an outfall? ☐ Yes ☐ No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

• Will organisms be released?

8. If applicable, how will the collected samples be transported out of the Monument?

Appendix C: Native Hawaiian Practices Application

1. Please state how the purpose and intent of the activity are appropriate and deemed necessary by traditional standards in the Native Hawaiian culture (pono), and demonstrate an understanding of, and background in, the traditional practice, and its associated values and protocols:

2. Please state how the activity benefits the resources of the Northwestern Hawaiian Islands and the Native Hawaiian community:

3. Please state how the activity supports or advances the perpetuation of traditional knowledge and ancestral connections of Native Hawaiians to the Northwestern Hawaiian Islands:

4. Will you be collecting any Monument resource? ☐ Yes ☐ No
If so, please provide the following information:

4a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name and/or Scientific name:

& size of specimens:

Collection location:

☐ Whole Organism ☐ Partial Organism

4b. What will be done with the specimens after the Native Hawaiian cultural practice is complete?

4c. Will organisms be kept alive after collection? ☐ Yes ☐ No

• Specific site/location:

• Is it an open or closed system? ☐ Open ☐ Closed

• Is there an outfall? ☐ Yes ☐ No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

• Will organisms be released?

NOTE: Any Monument resource harvested from the Monument for the purpose of Native Hawaiian practices will be consumed in the Monument.

5. Are you collaborating with others in any way to reduce duplicative activities in the Monument or elsewhere?

6. Gear and materials:

7. Will you erect any Native Hawaiian cultural structures or leave any offerings in the Monument? ☐ Yes ☐ No

If so, please describe:

8. Will you produce any publications, educational materials or other deliverables?
☐ Yes ☐ No

Provide a time line for write-up and publication of information or production of materials:

Appendix D: Recreation Application

For Activities in the Midway Atoll Special Management Area Only

- 1. Please explain how the activity is for the purpose of recreation as defined: An activity conducted for personal enjoyment that does not result in the extraction of Monument resources and that does not involve a fee-for-service transaction:**
- 2. Other Associated Monument Permits:**
- 3. Gear and materials:**
- 4. Fixed installations and instrumentation:**

Appendix E: Special Ocean Use Application

NOTE: If this is a first time Special Ocean Use activity, it will be subject to a pilot project.

1. Please provide proof of general liability insurance, or indicate that you will be posting an equivalent bond against claims arising out of activities conducted under the permit:

2. Are you collaborating with others in any way to reduce duplicative activities in the Monument or elsewhere?

3. Gear and materials:

4. Fixed installations and instrumentation:

5. List all publications directly related to the proposed project:

For projects occurring with the Midway Atoll Special Management Area answer the following questions:

6. Please explain how your activity has been found compatible with the purposes for which the Midway Atoll National Wildlife Refuge was designated?

7. Please explain how your activity meets the requirement of furthering conservation and management of the Monument:

For projects occurring outside of the Midway Atoll Special Management Area answer the following questions:

8. Please explain how the proposed activity will directly benefit the conservation and management of the Monument:

9. Please explain how the purpose of the proposed activity is for research and education related to resources or qualities of the Monument:

NOTE: SPECIAL OCEAN USE PERMITS OUTSIDE THE MIDWAY ATOLL SPECIAL MANAGEMENT AREA DO NOT ALLOW THE USE OF A COMMERCIAL PASSENGER VESSEL. A commercial passenger vessel is defined by the monument regulations as a vessel that carries individuals who have paid for such carriage.

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